

PATENT APPLICATION

METHOD FOR DIFFERENTIATING BREAST DUCTS FOR CANCER RISK STATUS

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METHOD FOR DIFFERENTIATING BREAST DUCTS FOR CANCER RISK STATUS

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BACKGROUND OF THE INVENTION

10 Benefit of the May 10, 2000 filing date of Provisional Application Serial No. 60/203,416 and benefit of the May 9, 2001 filing date of a Provisional Application (Serial No. not yet assigned), both provisionals by the same inventors and both entitled "Method For Differentiating Breast Ducts For Cancer Risk Status" are hereby claimed.

15 It is believed that breast cancer begins in the breast milk ducts and it is known that the human breast has from about 6 to 9 ducts. Emerging technology is providing systems, kits, methods and devices for collecting fluid from these ducts individually so as to keep separate the material collected from each duct and to analyze it separately. Copending and co-owned applications to methods and kits for obtaining fluid and cellular material from breast ducts, USSN 09/067,661 filed April 28, 1998, and its CIP USSN 09/301,058 filed April 28, 1999 describe and claim infusing fluid into the duct and collecting the fluid using a catheter.

20 Previously it has been found that breasts that yielded fluid upon nipple aspiration identified the patient as being at risk for developing breast cancer. See Petrakis, "Studies on the epidemiology and natural history of benign breast disease and breast cancer using nipple aspirate fluid" *Cancer Epidemiology, Biomarkers and Prevention* (Jan/Feb 1993) 2:3-10; Petrakis, "Nipple Aspirate Fluid in epidemiological studies of breast disease", *Epidemiologic Reviews* (1993) 15:188-195. In addition, pooled nipple aspirate fluid has been analyzed for cancer markers. See Sauter *et al*, "Nipple aspirate fluid: a promising non-invasive method to identify 25 cellular markers of breast cancer risk", *British Journal of Cancer* 76(4):494-501 (1997).

30 Upon identification of a duct that may have abnormal cells by analysis of the ductal fluid, that duct can be targeted for breast sparing treatment include administering an agent intraductally to the duct, rather than systemically, or removing just the affected duct. In order to review the entire breast by this method, however, all 6 to 9 ducts on the breast must be identified and accessed, which is a difficult and time consuming task, and for these reasons accessing all the ducts on a breast in order to perform a diagnosis remains a suboptimal strategy.

There is thus a need in the art to develop techniques to identifying ducts that are at higher risk for cancer than other ducts on the same breast so that fewer than all the ducts on the breast can be targeted for access and analysis. The present invention provides such a solution to this problem.

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SUMMARY OF THE INVENTION

1 It is an object of the invention to provide methods for differentiating a cancer risk status
2 of milk ducts in a breast.

3 It is another object of the invention to provide kits for differentiating a cancer risk status
4 of milk ducts in a breast.

5 These and other objects of the invention are provided by one or more of the embodiments
6 described below. In one embodiment a method of differentiating a cancer risk status of milk
7 ducts in a breast comprises aspirating the nipple, and locating at least one ductal orifice that
8 yields fluid upon aspiration; wherein a duct that yields fluid upon aspiration is at higher risk for
9 cancer. More than one duct can yield fluid at the orifice upon aspiration of the nipple. The
10 method can further comprise accessing a ductal orifice that yields fluid; retrieving ductal
11 contents from the accessed duct; recording the location of the ductal orifice once identified by
12 yield of fluid at the orifice; and/or making an identifiable mark at the ductal orifice upon yield of
13 fluid at the orifice. Recording can comprise one or more of transcribing the relative location of
14 the orifice onto a paper grid taking a photograph, recording in real time on a digital screen the
15 fluid yielding event and/or location of the ductal orifice that yielded fluid, and making a negative
16 imprint on the nipple surface to identify the regions of the nipple that did not yield fluid.

17 Marking can comprise making an identifiable mark with a pen or other labeling device to
18 identify the spot comprising the ductal orifice at a later time; and/or placing an element into the
19 duct such as a plug, tube, wire, thread or suture. The mark can reside on the nipple surface in a
20 range of time from a few hours to a few years. The method can further comprise contacting a
21 ductal orifice that yields fluid with a dilator in order to accomplish one or more of discerning the
22 precise location of the orifice, discerning the orientation of the orifice, or enlarging the proximal
23 area of the duct so as to facilitate subsequent cannulation of the duct.

24 Another embodiment of the invention provides a method of differentiating a cancer risk
25 status of milk ducts in a breast comprising aspirating the nipple, and locating at least one ductal

26 orifice that yields fluid upon aspiration; wherein a duct that yields fluid upon aspiration is at
27 higher risk for cancer; and collecting a bead of fluid at the nipple surface generated from
28 aspiration and emerging from the fluid yielding duct and not mixed with fluid generated from
29 any other fluid yielding ducts on the nipple surface. The method can further comprise analyzing
30 the collected fluid of the duct yielding fluid separately from the fluid of any other duct yielding
31 fluid; marking the duct that yields fluid; and/or recording the location of the ductal orifice once
32 identified by yield of fluid at the orifice. Recording can comprise one or more of transcribing
33 the relative location of the orifice on a paper grid, taking a photograph, recording in real time on
34 a digital screen the fluid yielding event and/or location of the ductal orifice that yielded fluid,
35 and making a negative imprint on the nipple surface to identify the regions of the nipple that did
36 not yield fluid. Marking can comprise making an identifiable mark with a pen or other labeling
37 device to identify the spot comprising the ductal orifice at a later time; and/or placing an element
38 into the duct such as a plug, tube, wire, thread or suture. The mark can reside on the nipple
39 surface in a range of time from a few hours to a few years. The method can further comprise
40 contacting a ductal orifice that yields fluid with a dilator in order to accomplish one or more of
41 discerning the precise location of the orifice, discerning the orientation of the orifice, or
42 enlarging the proximal area of the duct so as to facilitate subsequent cannulation of the duct

43 Embodiments including kits for differentiating a cancer risk status of milk ducts in a
44 breast can comprise a nipple aspiration device, a system to mark and/or record the location of an
45 orifice that yields fluid upon aspiration, and instructions for use of the kit to differentiate a
46 cancer risk status of milk ducts in a breast by aspirating the nipple and locating at least one
47 ductal orifice that yields fluid upon aspiration. Such a kit can further comprise a ductal access
48 tool and further instructions to access the duct that yields fluid upon nipple aspiration. The
49 system to mark and/or record the location of the ductal orifice that yields fluid can comprise one
50 or more of a pencil and paper grid, a camera, a marking tool, a digital recording and imaging
51 device, a system to make a negative imprint on the nipple surface, and an element to place in the
52 orifice to mark it. The kit may also comprise a dilator. Another kit for differentiating a cancer
53 risk status of milk ducts in a breast can comprise a nipple aspiration device, ductal access tool to
54 access a duct through an orifice that yields fluid upon aspiration, and instructions for use of the
55 kit to differentiate a cancer risk status of milk ducts in a breast by aspirating the nipple, including
56 instructions to locate at least one ductal orifice that yields fluid upon aspiration, and access that

57 duct through its orifice. Optionally the kit can also comprise a dilator.

58 Another kit for differentiating a cancer risk status of milk ducts in a breast can
59 comprise a nipple aspiration device, a tool to collect an emerging bead of fluid at a ductal orifice,
60 without mixing the collected fluid with any other fluid yielded from any other duct, and
61 instructions for use of the kit to differentiate a cancer risk status of milk ducts in a breast by
62 locating at least one ductal orifice upon aspiration of the nipple and instructions to collect a bead
63 of fluid from that ductal orifice at the nipple surface without mixing the collected fluid with any
64 other fluid yielded from any other duct on the nipple surface. Such a kit can further comprise
65 means and instructions for analyzing any collected ductal fluid for making a determination of
66 cancer risk in that duct. All kits can further comprise a container for the kit contents.

67 It is an object of the invention to maximize the likelihood of ductal fluid migrating to the
68 nipple surface upon nipple aspiration. Accordingly, there is provided a method of maximizing
69 the likelihood of ductal fluid migrating to the nipple surface upon nipple aspiration comprising
70 stimulating the breast and/or nipple surface prior to or during nipple aspiration. Stimulating can
71 comprise placing a wearable device in contact with the nipple surface.

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DETAILED DESCRIPTION OF THE EMBODIMENTS

It is a discovery of the invention that breast ducts on a human breast nipple which are at 5 higher risk for cancer can be differentiated from those ducts which are at lower risk. After identification of the higher risk ducts these ducts can then be marked and/or their location recorded e.g. for purposes of facilitating subsequent location and access. Marking can facilitate 10 locating the higher risk duct or ducts later for access, treatment or monitoring. The higher risk ducts can be accessed and their ductal contents analyzed for abnormal cells or other markers that would indicate cancer or cancer precursors. If cancer is identified in a duct, it is marked and can 15 be treated and/or monitored. Thus, differentiating a cancer risk status of breast ducts of a breast includes determining which of the 6 to 9 breast ducts on the breast is most or more likely to have carcinoma or other cancer or the precursors of carcinoma or other cancer, and therefore should be analyzed for the presence of cancer marker in the contents of the ductal fluid.

Breast cancer is believed to originate in the lining of a single breast milk duct in the 15 breast; and additionally human breasts are believed to contain from 6 to 9 of these ducts. See Sartorius, *JAMA* 224 (6): 823-827 (1973). It is now generally believed that the human breast has from 6 to 9 milk ducts which begin at the ductal orifices on the nipple surface and which 20 extend and branch in the breast until the ductal lumens reach and become the terminal ductal lobular unit (TDLU) at the base of the breast. Breast cancer is believed to begin in the milk ducts, and the ductal carcinoma or carcinoma in the TDLU can be *in situ* or the carcinoma can be invasive. In the early stages of breast cancer it is likely that not all 6 to 9 ducts are affected but rather a duct or a number of ducts something less than the total number of ducts have carcinoma 25 and the rest are unaffected. As such it would be advantageous to the medical community and patient population to be able to identify the ducts that are more likely to have carcinoma, and from which ductal fluid should be collected for analysis.

The method is practiced by aspirating the nipple on the target breast. Nipple aspiration has been used to retrieve ductal fluid for analysis of fluid pooled from all or any yielding ducts in the target breast. Nipple aspiration can be accomplished by placing an aspiration cup on the 30 nipple and generating a negative pressure or vacuum on the nipple surface to encourage any fluid within the ducts to come to the surface of the nipple. The aspiration device can resemble a

breast pump used by lactating mothers to retrieve breast milk. Aspiration can be accomplished essentially as described in co-pending and co-owned patent application filed November 12, 1999, docket 99-023. Aspiration can also be accomplished as described in Papanicolaou et al, "Exfoliative Cytology of the Human Mammary Gland and Its Value in the Diagnosis of Cancer and Other Diseases of the Breast" *Cancer* (1958) March/April 377-409 Papanicolaou et al (1958) 5 *Cancer*, 11:377-409; Goodson WH & King EB, *Chapter 4: Discharges and Secretions of the Nipple*, *The Breast: Comprehensive Management of Benign and Malignant Diseases* (1998) 2nd Ed. vol 2, Bland & Kirby eds. W.B. Saunders Co, Philadelphia, PA pp. 51-74; Sartorius et al (1977) *Journal of the National Cancer Institute* 59(4):1073-80; King et al, (1983) *Journal of the 10 National Cancer Institute* 71(6):1115-21; Wrensch et al, (1992) *Am. J. Epidemiology*, v. 135 (2): 130-141. See also generally, *The Breast: Comprehensive Management of Benign and Malignant Breast Diseases*, Bland and Copeland eds. W.B. Saunders Co. Philadelphia PA 1991 15 pages 61-67. The nipple aspiration process can be repeated several times until fluid is yielded. In addition, fluid yield may be facilitated by massaging and/or squeezing the breast before or during the aspiration, or by other techniques such as the application of heat to the breast before or during the aspiration procedure.

Nipple aspiration will result in yield of fluid at at least one duct on the nipple surface in some women. During the process of locating a ductal orifice that yields fluid upon aspiration, it is important to identify the small amount of fluid coming from the ductal orifice as early in the 20 aspiration procedure as possible. In this way, the droplet or bead developing at the particular orifice is linked directly to the duct yielding the fluid and not confused with any other ducts that are also yielding fluid or any other topography on the nipple surface that could be mistaken for an orifice. The location on the nipple surface from which the fluid first comes forward to the surface is presumed to be the location of the ductal orifice on that nipple surface. The nipple 25 surface has an uneven topography with much layered skin and other contours so that orifice identification can be difficult without the presence of an indicator such as a small unit of fluid that is perhaps a bit shinier than the nipple surface and therefore distinguishable from the rest of the surface that is not yielding fluid. More than one duct can yield fluid on the nipple surface during a nipple aspiration procedure. In that case, an opportunity is presented to locate more 30 than one duct by identifying the ductal orifice at which the fluid is appearing or emerging. More than one or multiple ductal orifices can yield fluid during aspiration, be marked and/or recorded

for the location of the orifice, and/or be accessed as described herein.

As such the location can be recorded or marked or otherwise identified at the first sign of fluid yield. One simple means of identifying the location where the fluid first comes onto the nipple surface is to use the naked eye, or a magnification tool such as a loupe or microscope.

5 Once it is believed that the ductal orifice is located, since it would be easy to forget or lose track of the exact position of the orifice, the nipple surface can be marked, or the location of the orifice can be otherwise recorded for re-identification of the location of the orifice later.

Marking can be making an identifiable mark on the nipple surface, e.g. with a pen or other marker. The mark on the nipple surface can be read using visible light, or may be UV light

10 sensitive, etc. Recording the location of the orifice can be accomplished for example, by marking the relative position of the orifice on graph paper or other grid using a pencil or pen, for example orienting the breast and nipple on a north-south-east-west axis and marking the location of the orifice relative to these axis and/or other landmarks on the breast, nipple or patient.

Marking can also be accomplished by marking by taking a photograph of the nipple (e.g. before, 15 after and during fluid yield), or recording the process of fluid yield in real time with an analog or digital video camera so that the fluid yield is documented and recorded on a digital screen.

Additionally, for example, a negative imprint on the nipple surface can be made to identify the regions of the nipple that did not yield fluid, in order to particularly highlight the very small regions that did yield fluid. Marking the ductal orifice can also be accomplished by placing an 20 element in the duct once it is identified, e.g. a plug, tube, wire, thread or suture, etc into the duct.

The element can be seen at the nipple surface to identify the orifice later. Also, having a physical element in the duct can be facilitate ductal access later if desired, for example a tube that can provide a channel for accessing the duct, or a wire that keeps the duct open until the practitioner can access it.

25 In any event the mark made at the orifice or around the orifice is an identifiable mark made so that the orifice can be identified at some time later. For example, one or more orifices yield fluid upon aspiration and are marked. Each one can then be accessed in turn and fluid retrieved from each duct is kept separate and analyzed separately. The identifiable mark can be made with a pen (e.g. a pen for human skin) or other labeling device to identify the spot on the 30 nipple surface at which the ductal orifice is located. The labeling can last a short time or a long time depending on the needs of the practitioner and/or the patient. For example, the mark can

remain until washed off with water or alcohol or other effective solvent (e.g. about an hour or two or a half or full day) or can act like a semi-permanent tattoo and remain on the skin for a about year or two or more.

In any event, whether the marking lasts for a short period of time or a longer period of time, the position or location of the ductal orifice can be recorded. The position or location can be relative to the topography of the nipple surface, a north-west-south east orientation or other landmarks on the body which can help identify the location of a tiny spot on the skin surface. Recording the location of the ductal orifice can be useful, e.g. to watch or monitor the duct, to facilitate treatment of an affected or suspect or high risk duct (e.g. affected with cancer or suspicious for cancer, or at high risk for cancer or cellular abnormality), or simply to retain a diagnostic or therapeutic history of that duct. Treatment of a duct can include e.g. accessing a duct and/or removing all or a portion of it, or removing all or a portion of the epithelial lining of the duct.

After an orifice yields fluid upon nipple aspiration, the orifice can be contacted with a dilator (for example a dilator used in galactography procedures) in order to discern the precise location of the duct, discern the orientation of the duct (i.e. does it go straight in or in an angle), or to enlarge the orifice, e.g. by replacing the duct with dilators of successively larger diameter in order to dilate the orifice and early portion of the duct, so that access with an access tool might be easier.

The discovery of the invention is also that a nipple surface can be aspirated and at the first appearance of fluid, met with an action to collect the emerging fluid from the nipple surface before the fluid mixes with other fluid from other ducts. The fluid bead can be collected or retrieved by any suitable means or tool for doing so, e.g. with a small tube that pulls the fluid into it, e.g. a capillary tube. The fluid may be also collected, e.g. with an absorbant material that contacts the emerging bead and absorbs it. The importance of collecting the bead as it emerges from the orifice and not after the bead has mixed with fluid from any other orifice is that the fluid can be attributed to a single duct and as such can be analyzed independently of any other ducts on the breast. Thus, what can follow in the procedure once the nipple is aspirated and a fluid bead emerges and is collected from an orifice before it mixes with fluid from other ducts, is that the fluid can be analyzed and/or the ductal orifice where the bead emerges can be marked or the location of the orifice can be otherwise recorded as described herein for the purpose of

keeping track of the status of the duct. For example, if the analysis of the ductal fluid indicates cancer or precancer a patient or clinician may choose to treat and/or monitor the duct and it would then be essential to be able to relocate with certainty the same duct from which the analyzed fluid was retrieved.

5 If the orifice and duct is accessed, the ductal contents may be retrieved from within the duct through the tool accessing the duct, and collected for analysis. A detection of abnormality in the cells, or of a particular marker or markers present in the ductal contents that indicate a warning sign for cancer, or detection of carcinoma itself may result from the analysis. In any event, the located and marked duct can be treated and/or monitored subsequent to the finding of
10 high risk or other diagnosis.

The invention also provides a kit for differentiating a cancer risk status of breast ducts on a nipple comprising a nipple aspiration device (e.g. any of those described herein), and a marking and/or recording system to mark or record the location of any orifice that yields fluid from the nipple surface upon aspiration. The kit will contain instructions for use of the kit to
15 differentiate breast ducts on a nipple by cancer risk status using nipple aspiration to find a duct or ducts which yield fluid upon aspiration. The kit can further comprise a ductal access tool, and instructions for accessing a fluid yielding duct identified by the nipple aspiration procedure. The kit can also contain one or more dilators (for example galactography dilators) for dilating a ductal orifice before accessing the duct with a ductal access tool.

20 Alternatively the kit can comprise a nipple aspiration device and a ductal access tool to access the duct and collect fluid for analysis. This kit will also contain instructions for aspirating the nipple to locate the ducts at risk, and accessing the ducts that yield fluid upon nipple aspiration. The kit can also contain the marking and/or recording tools mentioned herein. The kit can contain a nipple aspiration device and a means or tool to collect an emerging bead of
25 fluid off the nipple surface without mixing the fluid with fluid from any other duct on that nipple surface. This kit can also contain marking and/or recording tools for marking the location of ductal orifices so identified. The kit may also contain one or more dilators: for example a galactography dilator or dilators. The kit will contain instructions for aspirating and collecting as described herein. Any of the kits can have a marking and/or recording system, e.g. to locate
30 any orifice that yields fluid upon aspiration, for example a camera, marking tool (e.g. a pen or tattoo making tool), a digital recording device, a digital imaging device, and/or a system to make

a negative imprint on the nipple surface, and/or an element to place in the orifice and duct to mark it (e.g. a plug, wire or tube). Thus also any of the kits may contain a nipple aspiration device and a ductal access tool, and any of the kits may contain a nipple aspiration device and a tool to collect an emerging bead of fluid at the ductal orifice, e.g. a tube (e.g. a capillary tube), or 5 a tool having an absorbant tip or an absorbant material that can contact the bead and absorb it. .

The kits of the invention can further have a container for the kit contents.

Sensitivity for differentiating a cancer risk status of milk ducts by aspirating the nipple can be further provided by maximizing the likelihood that ductal fluid already present in the duct will migrate to the nipple surface upon aspiration of the nipple. Maximizing the likelihood of 10 migration of ductal fluid to the nipple surface can be accomplished by stimulating the breast and/or nipple surface. Stimulation of the breast and/or nipple surface can be accomplished e.g. by providing vibration at the nipple surface or at the breast as a whole. Vibration can be accomplished e.g. by placing a wearable device in contact with the nipple surface or breast for a period of time. The wearable device can provide continuous or intermittent vibration to the 15 nipple or breast while being worn. The vibration can be provided for example by battery driven energy, or by a microchip controlled device. The device can be for example a bra-like item, or a patch affixed to the nipple.

The following examples are offered by way of illustration, not by way of limitation.

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EXAMPLES

Example 1.

A mammographically negative and physical exam negative woman was prepared for nipple aspiration on both her breasts. First the left nipple was aspirated, resulting in yield of a small amount of fluid from a ductal orifice at the nipple surface. The fluid was collected with a 25 capillary tube without mixing the fluid with other portions of the nipple surface, and a small pen mark was placed at the site of the fluid yield and ductal orifice. The nipple was photographed, and the position of the ducts were marked on a grid. The duct yielding fluid was accessed subsequently and more ductal fluid was collected from within the duct. The nipple aspirate fluid collected in the capillary tube and the fluid collected from within the duct were analyzed 30 separately for cancer markers and abnormal cell morphology. The procedure was repeated on the right breast in which two ductal orifices yielded fluid. The fluid was collected separately

with a capillary tube, and the ductal orifices marked. The positions of the orifices were recorded on a grid, and the orifices were marked with a pen on the nipple surface. The ducts were accessed sequentially, and ductal fluid was retrieved from within the ducts and analyzed separately from each other and from the fluid collected in the capillary tubes.

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Example 2

A female patient undergoes nipple aspiration on both breasts. The right breast yields two droplets at different locations on the nipple surface. The nipple surface at the droplets is marked with a skin marking pen (e.g. sharpie brand fine tip marker). Location B is marked with pen 10 also, perhaps using a different color of sharpie (e.g. red or blue). Location A is proved with a ductal access tool (e.g. a catheter) and saline is infused into the duct. After the breast is massaged, ductal fluid is retrieved in the lumen of the catheter and collected in a collection tube 15 using gentle aspiration at the lumen. The procedure is repeated at location B. The left breast yielded one droplet (location C) which is marked also with a sharpie pen, and the procedure of ductal access, fluid infusion, and withdrawal is repeated on the left breast.

Material (e.g. fluid, cells and/or other markers) from each duct A, B, and C is analyzed by cytology and for breast cancer specific markers in order to identify with precision the risk status of each duct for cancer or precancer, and ultimately to evaluate each breast of the patient for the presence of absence of breast cancer or precancer.

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All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of 25 understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.